

Essential Oil Composition of Menthol Mint (*Mentha arvensis*) and Peppermint (*Mentha piperita*) Cultivars at Different Stages of Plant Growth from Kumaon Region of Western Himalaya

R.S. Verma^{*1,2}, L. Rahman³, R.K. Verma^{1,2}, A. Chauhan^{1,2}, A.K. Yadav^{1,2} and A. Singh¹

¹Central Institute of Medicinal and Aromatic Plants, Resource Centre, Purara, P.O.- Gagrigole, Bageshwar, Uttarakhand- 263688, India.

²Central Institute of Medicinal and Aromatic Plants, Resource Centre, Pantnagar, P.O. - Dairy farm Nagla, Udham Singh Nagar, Uttarakhand- 263149, India

³Central Institute of Medicinal and Aromatic Plants, P.O. - CIMAP, Lucknow - 226015, India

*Corresponding author. rswaroop1979@yahoo.com ²Present address

Abstract: Menthol mint (*Mentha arvensis* L.) and peppermint (*M. piperita* L.) cultivars grown in Kumaon region were evaluated for essential oil content and composition at different stages of crop growth. In menthol mint cultivars viz., 'Kosi', 'Saksham', 'Himalaya', and 'Kalka', the essential oil content was found to vary from 0.3% - 1.2%, 0.42% - 1.1%, 0.38% - 1.0% and 0.26% - 1.2%, at different days after transplanting (DAT) respectively, while in cultivars 'Kukrail', 'CIM-Madhurus' and 'CIM-Indus' of peppermint, it varied from 0.28% - 0.6%, 0.19% - 0.55% and 0.17% - 0.37%, respectively at different DAT. The menthol content in all the menthol mint cultivars reached higher values at 120 and 150 DAT. In case of peppermint cultivars viz., 'Kukrail', 'CIM-Madhurus' and 'CIM-Indus', menthol content varied from 32.92% - 39.65%, 34.29% - 42.83% and 22.56% - 32.77%, respectively during the crop growth.

Key words: *Mentha arvensis* L., *Mentha piperita* L., cultivars, crop age, essential oil composition

Introduction

Mints comprise a group of species of the genus *Mentha* belonging to the family Lamiaceae. The aerial parts of the herb on distillation yields essential oil containing a large number of aroma chemicals like menthol, menthone, isomenthone, menthofuran, carvone, linalool, linalyl acetate and piperitenone oxide which are used in pharmaceutical, food, flavour, cosmetics, beverages and allied industries. Mint oils are mainly produced in Argentina, Angola, Australia, Brazil, Bulgaria, China, Czechoslovakia, France, Hungary, India, Italy, Paraguay, Switzerland, Thailand and USA. In India, mint species are mainly cultivated in Uttar Pradesh, Haryana and Punjab and they occupied more than 162800 hectares of agricultural land. The annual world production of *Mentha arvensis* L. and *M. piperita* L. oils are 22,000 and 7500 mt, while India is producing 16000 and 100 mt per year, respectively (Khanuja, 2007; Patra *et al.*, 2002).

Like in any other aromatic crop, the yield and the essential oil composition of mint species are influenced by interaction between the genotype and environment, method of distillation, kind of storage, crop age, time of harvest, and season (Chalchat *et al.*, 1997; Farooqi *et al.*, 1983; Gasic *et al.*, 1989; Kofidis *et al.*, 2004; Kumar *et al.*, 2000; Kumar *et al.*, 1999; Lawrence, 1981, 1989, 1993, 1994; Marotti *et al.*, 1993; Rao *et al.*, 1999; Srivastava *et al.*, 2000). The time of harvest, in general has a close relation to yield and quality of oil and it varies from

place to place and genotype to genotype. Many experiments have been conducted on mint species in warm climates, but few studies have so far been published from sub temperate region (Bharadwaj and Srivastava, 1984). This prompted us to examine the effects of crop age on the content and quality of essential oils of seven genotypes of two commercially cultivated mint species viz., *M. arvensis* and *M. piperita* under hill conditions of Uttarakhand.

Materials and methods

Plant Material

The experiment was conducted during 2006 to 2008 (two cropping season) using cultivars 'Kosi', 'Saksham', 'Kalka' and 'Himalaya' of menthol mint (*M. arvensis*) and 'Kukrail', 'CIM-Madhurus' and 'CIM-Indus' of peppermint, *M. piperita*. The peppermint cultivars were planted in the month of January and menthol mint cultivars in March at an inter row space of 50 cm. All cultivars were raised following normal agricultural practices at the experimental farm of the Central Institute of Medicinal and Aromatic Plants (CIMAP), Resource Centre, Purara, Bageshwar. The experimental site is located at an elevation of 1250 m having sub-temperate mild climate. Sampling was started 30 days after transplanting (DAT) of stolon and samples were taken every month at an interval of 30 days for extraction of essential oil. However, in case of cultivar 'CIM-Indus' sampling was started at 60 DAT onwards due to non-availability of sufficient herbage at 30 days.

Extraction of Essential Oil

Freshly harvested samples of all cultivars were hydro-distilled in a Clevenger's apparatus for 3 hrs for extraction of essential oil. The oils were collected, dehydrated by anhydrous sodium sulphate, measured and kept in a cool and dark place prior to analysis.

Gas Chromatography (GC)

The oil samples were subjected to GC analysis (Nucon gas chromatograph model 5765 equipped with FID) using fused silica capillary column (30 m x 0.25 mm x 0.25 µm film thickness) and stationary phase BP-20 (coated with a carbowax 20M). Hydrogen was used as a carrier gas at the rate of 1.0 ml/min. Injector and detector temperatures were 200°C and 230°C, respectively. The column temperature was programmed from 70°C to 230°C at 4°C/min, with the initial hold time of 2 min. The injection volume was 0.02 µl neat.

Identification of Compounds

The identification was done by comparing the retention time, retention indices and mass fragmentation pattern of the peaks with literature or standard compounds run under the same conditions and by peak enrichment on co-injection with authentic samples wherever possible to corroborate identities. The peak area percentage was computed from the peak areas without applying FID response factor correction.

Results and Discussion

The essential oil content and terpenoids composition of menthol mint and peppermint cultivars were found to vary with respect to crop age (Table 1, 2, 3, 4). In menthol mint cultivars viz., Kosi', 'Saksham', 'Himalaya', and 'Kalka' the essential oil content at different DAT was found to vary from 0.35% - 1.2%, 0.42% - 1.1%, 0.38% - 1.0% and 0.26% - 1.2%, respectively, while in cultivars 'Kukrail', 'CIM-Madhurus' and 'CIM-Indus' of peppermint it varied from 0.28% - 0.6%, 0.19% - 0.55% and 0.17% - 0.37%, respectively. Further, in case of cultivars 'Kosi' and 'Himalaya', maximum oil content was obtained from the crop harvested after 120 DAT and it remained consistent up to 150 DAT. On the other hand, in cultivars 'Saksham' and 'Kalka', oil content was higher when the crop was harvested at 150 DAT as compared to the other crop durations. The two peppermint cultivars viz., 'Kukrail' and 'CIM-Madhurus' were also found to have maximum oil content after 150 DAT (0.60% and 0.55%). However, in cultivar 'CIM-Indus' the essential oil content reached higher value at 180 DAT (0.37%). Similar trend of essential oil accumulation in these two mint species was reported under climatic conditions of north Indian plains (Bahl *et al.*, 2000; Kumar *et al.*, 2000).

The essential oils obtained from menthol mint and peppermint cultivars at different crop age were

Table 1: Chemical profile of menthol mint (*Mentha arvensis*) cultivars at different stages of crop growth from Uttarakhand hills

Compound	Age of plant (DAT) in Cv. 'Kosi'					Age of plant (DAT) in Cv. 'Saksham'				
	30	60	90	120	150	30	60	90	120	150
α-pinene	t	0.91	0.47	1.40	1.01	0.41	0.38	0.61	0.72	0.46
β-pinene	t	0.78	0.56	0.94	0.30	0.85	0.53	0.79	0.74	0.65
sabinene	t	t	t	t	t	t	t	t	0.11	t
β-myrcene	t	0.49	0.40	0.56	0.51	t	0.24	0.40	0.39	0.43
limonene	2.48	4.74	1.57	2.21	1.74	3.23	3.81	0.98	1.06	2.26
1,8 cineole	-	-	t	t	t	-	t	t	t	t
(E)-β-ocimene	t	-	-	-	-	-	-	-	-	-
(Z)-3-hexenol	-	t	-	-	-	-	-	-	-	-
3-octanol	t	1.79	1.61	1.45	1.61	0.74	1.50	4.24	0.83	2.54
menthone	19.32	7.67	7.46	3.60	5.53	11.32	6.98	6.53	7.64	5.40
(E)-sabinene hydrate	t	-	-	-	-	-	-	t	t	-
iso-menthone	3.46	3.32	3.56	2.88	3.24	2.65	2.94	3.48	3.32	3.49
linalool	0.38	t	t	0.20	t	t	-	-	t	t
menthyl acetate	0.58	3.18	1.12	2.53	1.34	3.27	4.41	1.44	1.86	0.81
isopulegol	1.57	1.16	1.01	1.27	1.34	1.03	1.04	0.94	0.83	0.81
β-caryophyllene	t	-	-	t	t	-	-	-	-	t
neo-menthol	1.91	1.91	1.93	1.82	1.64	1.53	1.92	1.74	1.61	2.31
terpinen-4-ol	t	0.12	0.65	0.28	0.67	0.42	0.30	0.33	0.13	0.37
pulegone	t	t	t	t	t	t	0.47	0.34	0.49	0.35
menthol	61.92	71.90	77.54	78.21	77.16	70.47	72.64	75.85	76.06	76.11
isomenthol	-	-	0.15	0.47	0.18	t	t	0.11	-	-
α-terpineol	-	-	-	t	0.18	-	0.10	0.17	0.17	0.52
germacrene-D	t	0.16	0.16	0.25	0.29	0.32	0.11	t	t	0.18
piperitone	0.83	0.53	0.24	0.46	0.64	0.61	1.22	1.76	1.56	0.53
carvone	-	t	-	-	-	t	t	-	-	0.16
Oil content (%)	0.35	0.66	1.10	1.20	1.20	0.42	0.52	0.82	1.0	1.10

DAT= days after transplanting

t= trace (<0.1%)

Table 2: Chemical profile of menthol mint (*Mentha arvensis*) cultivars at different stages of crop growth from Uttarakhand hills

Compound	Age of plant (DAT) in Cv. 'Himalaya'					Age of plant (DAT) in Cv. 'Kalka'				
	30	60	90	120	150	30	60	90	120	150
α -pinene	0.37	0.85	0.55	0.69	0.21	-	0.60	1.25	0.90	0.62
β -pinene	0.89	0.88	0.24	0.71	0.08	-	0.80	0.86	0.40	0.81
sabinene	t	0.12	0.14	0.12	t	-	t	t	t	t
β -myrcene	-	0.61	0.31	0.27	0.36	-	0.45	0.49	0.55	0.45
limonene	3.49	4.51	0.67	0.66	1.73	0.27	3.74	1.85	1.41	1.34
1,8 cineole	-	t	0.11	t	t	-	-	-	t	t
(E)- β -ocimene	-	-	t	t	-	t	-	-	-	-
(Z)-3-hexenol	-	-	-	-	-	-	t	-	-	-
3-octanol	0.44	3.30	1.73	2.20	0.90	-	1.09	t	1.27	2.16
1-octen-3-ol	-	-	-	-	t	-	-	-	-	-
menthone	14.02	6.27	5.08	3.43	6.37	8.11	7.58	11.85	3.80	5.89
(E)-sabinene hydrate	-	-	t	t	-	-	-	-	t	-
iso-menthone	3.17	2.60	3.16	3.08	3.62	2.36	3.06	6.09	3.18	4.30
linalool	t	t	-	-	0.48	-	t	-	-	-
menthyl acetate	0.60	4.00	1.63	1.62	0.69	3.07	2.93	3.67	3.53	0.54
isopulegol	0.74	0.92	0.73	0.92	0.49	0.87	1.16	1.16	1.23	1.38
β -caryophyllene	0.14	-	-	-	t	-	-	-	-	t
neo-menthol	1.35	2.08	1.61	1.68	1.43	2.41	1.95	1.45	1.73	1.63
terpinen-4-ol	0.28	0.48	0.31	0.41	0.34	-	t	0.40	0.13	0.50
pulegone	-	t	t	t	t	-	t	t	0.77	t
menthol	70.66	71.29	79.64	82.18	80.29	73.55	74.68	65.61	74.24	76.48
isomenthol	-	-	t	0.11	t	t	0.21	t	0.15	0.10
α -terpineol	-	t	0.17	0.34	0.61	-	-	0.18	0.16	0.19
germacrene-D	0.54	0.34	0.14	0.12	0.13	-	0.20	0.31	0.22	0.23
piperitone	1.43	0.50	1.08	0.98	0.96	0.90	0.55	2.05	0.66	0.88
carvone	-	0.12	-	-	-	-	t	-	-	-
Oil content (%)	0.38	0.45	0.75	1.0	1.0	0.26	0.52	1.0	1.1	1.2

DAT= days after transplanting

t= trace (<0.1%)

analysed by capillary gas chromatography. A total of 26 and 33 compounds were identified in oils of menthol mint and peppermint, respectively. Menthol mint oils were mainly composed of menthol, menthone, isomenthone, menthyl acetate, limonene and neomenthol. The menthol content was found to increase with the increase of crop age and it reached to peak during 120 to 150 DAT in all the menthol mint cultivars except in 'Kalka' where it showed some irregular trend. Similar menthol accumulation pattern in different genotypes of menthol mint was reported earlier (Anonymous, 2004 b). Menthone content was found to be higher at 30 DAT in cultivars 'Kosi', 'Saksham' and 'Himalaya' (19.32%, 11.32% and 14.02%), while in cultivar 'Kalka' it was found maximum in 90 DAT (11.85%). An increase in menthol content up to 99 days of crop age with decrease in menthone content was also reported (Singh *et al.*, 1988). Similarly, Randhawa and Satinder (1996) reported that the menthol in cornmint (menthol mint) increased but the menthone content decreased with delay in harvesting from 120 to 150 DAT which is in agreement with the results of cultivar 'Saksham'. Isomenthone concentration was higher at 90 DAT in Kosi (3.56%) and Kalka (6.07%) and at 150 DAT in 'Saksham' (3.49%) and 'Himalaya' (3.62%). Further, the percentage of menthyl acetate was high-

er at 60 DAT in cultivar 'Kosi', 'Saksham' and 'Himalaya' (3.18%, 4.41% and 4.0%), whereas at 90 DAT in cultivar 'Kalka' (3.67%). Interestingly, the limonene percentage was found maximum at 60 DAT in all the cultivars of menthol mint.

Although the qualitative composition of peppermint cultivars was almost similar to that of menthol mint, there were considerable variations in their quantitative compositions. The menthol content at different DAT varied from 32.92% - 39.65% in cultivar 'Kukrail' with the maximum at 30 DAT followed by crops of 150 DAT. However, in cultivar 'CIM-Indus' it was found higher at 120 DAT (32.77%) followed by 180 DAT (32.12%) while during rest of the time it ranged between 22.56% and 27.97%. On the other hand, in cultivar 'CIM-Madhurus' menthol percentage ranged between 34.29% (at 150 DAT) and 42.83% (60 DAT) during the period under study. The concentrations of menthyl acetate (30.70% and 32.80%), isopulegol (1.4% and 1.54%), pulegone (1.15% and 1.3%) and isomenthol (0.60% and 1.16%) were higher at 30 DAT in cultivars 'Kukrail' and 'CIM-Madhurus', respectively. However, the concentration of menthyl acetate and isopulegol was higher at 60 DAT in 'CIM-Indus'. According to earlier reports menthol and menthyl acetate concentrations increase

Table 3: Chemical profile of peppermint (*Mentha piperita*) cultivars at different stages of crop growth from Uttarakhand hills

Compound	Age of plant (DAT) in Cv. 'Kukrail'						Age of plant (DAT) in Cv. 'CIM-Madhurus'					
	30	60	90	120	150	180	30	60	90	120	150	180
α -pinene	0.50	0.58	0.91	0.85	0.94	0.82	0.44	0.46	1.00	0.86	0.88	0.78
β -pinene	0.31	0.86	0.55	1.33	1.29	1.48	0.24	0.35	1.48	1.13	1.37	1.59
sabinene	t	0.14	-	0.24	0.26	0.29	t	0.12	0.22	0.24	0.35	0.30
β -myrcene	-	0.14	0.24	t	0.24	0.28	-	-	0.26	t	t	t
α -terpinene	t	-	0.21	t	0.17	0.21	t	0.29	0.18	t	0.74	0.29
limonene	1.03	1.00	2.37	2.45	1.35	1.68	0.72	0.54	1.54	1.43	1.22	1.12
1,8 cineole	2.25	3.98	5.58	5.48	5.79	7.26	2.51	3.86	6.57	5.62	7.04	6.57
γ -terpinene	0.20	-	0.55	0.39	0.45	0.55	t	t	0.35	0.35	0.59	0.53
(<i>E</i>)- β -ocimene	0.23	0.68	0.30	0.20	0.33	0.16	t	0.87	1.29	1.69	0.38	0.47
<i>p</i> -cymene	t	t	0.37	0.20	t	t	t	t	0.18	-	0.21	t
3-octanol	-	t	t	1.23	t	0.50	-	t	t	0.29	0.65	0.53
menthone	8.09	31.11	29.83	22.87	33.77	23.64	3.46	25.57	26.88	17.12	29.81	29.67
menthofuran	0.21	0.43	0.81	2.02	2.41	4.99	-	0.29	0.32	0.42	1.05	3.96
iso-menthone	0.69	2.63	3.36	2.94	3.72	3.20	0.65	2.66	2.65	2.51	3.63	3.98
linalool	-	0.24	0.19	t	0.35	0.44	-	0.21	0.26	0.16	0.38	0.53
menthyl acetate	30.70	6.58	5.18	7.99	1.85	3.01	32.80	4.30	7.23	9.46	2.52	2.34
isopulegol	1.40	t	0.13	0.15	0.38	0.26	1.54	-	0.13	t	0.30	0.18
β -caryophyllene	0.19	0.79	0.96	0.95	0.74	1.03	-	0.89	1.10	0.89	0.79	1.02
neo-menthol	4.00	2.83	3.47	3.50	2.74	2.73	4.34	4.00	3.39	4.86	3.06	2.10
terpinen-4-ol	0.26	0.69	0.51	0.48	0.72	0.71	0.18	0.27	1.21	1.44	0.57	0.70
pulegone	1.15	0.69	0.68	0.79	0.53	0.66	1.30	0.28	0.96	1.23	0.70	0.63
menthol	39.65	32.92	37.44	37.28	34.54	38.81	42.30	42.83	36.13	40.53	38.42	34.29
(<i>E</i>)- β -farnesene	-	0.19	0.18	0.22	0.14	0.17	-	0.28	0.21	t	0.18	t
isomenthol	0.60	0.21	0.19	0.21	0.12	0.19	1.16	0.32	0.25	0.76	0.19	t
α -terpineol	-	0.28	0.16	0.33	0.16	0.32	-	t	0.16	t	0.36	0.79
germacrene-D	t	0.23	0.78	0.26	0.60	0.71	-	t	0.69	0.90	0.55	0.62
pipéritone	0.79	0.58	1.27	1.21	2.20	1.28	t	0.20	0.83	0.94	0.52	1.01
Oil content (%)	0.28	0.34	0.48	0.50	0.60	0.55	0.19	0.21	0.32	0.35	0.55	0.52

DAT= days after transplanting

t= trace

as the peppermint plant matures until about the flowering stage (Court *et al.*, 1993; Croteau and Loomis, 1972; Hefendehl *et al.*, 1973). This is not consistent with the results obtained in this study. Menthone and isomenthone contents were found to be higher at 150 DAT in 'Kukrail' and 150 and 180 DAT in 'CIM-Madhurus', respectively. However, Court *et al.*, (1993) reported that the concentration of these two constituents decreased with the advancement of plant stage. Menthofuran and β -myrcene were found in fairly good percentage (7.45%- 17.87% and 2.96%- 15.56%) in cultivar 'CIM-Indus' when compared to the other peppermint cultivars. The concentration of menthofuran was almost same at 60 and 180 DAT in this cultivar while during the rest of the time it varied from 7.45% to 16.27%. This is not in agreement with the finding from north Indian plain where menthofuran was 27.24% at 115 days old crop of 'CIM-Indus'(Anonymous, 2004 a). It is interesting to note that the amount of menthofuran increased with increase of crop age and it was highest at 180 DAT in cultivars 'Kukrail' and 'CIM-Madhurus'. 1, 8 cineole percentage was higher at 180 DAT

(7.26%) in 'Kukrail', at 150 DAT (7.04%) in 'CIM-Madhurus' and at 90 DAT in 'CIM-Indus' while during the rest of the time it varied from 2.25% - 5.79%, 2.51% - 6.57% and 6.90% - 13.94%, respectively. Other minor and trace constituents of these oils also showed considerable variation in their quantitative composition due to crop age.

The essential oil obtained from different harvesting times thus showed considerable variation in content and composition in both the mint species. Finally, considering these results it is concluded that the peppermint and menthol mint cultivars should be harvested in June (150 DAT) if the crop is sown in January and July/August (120 to 150 DAT) if the crop is sown in March, respectively, under the climatic conditions of Kumaon Region of Western Himalaya in Uttarakhand.

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Table 4: Chemical profile of peppermint (*Mentha piperita*) cultivar 'CIM-Indus' at different stages of crop growth from Uttarakhand hills

Compound	Age of the plant (DAT) in Cv. 'CIM-Indus'				
	60	90	120	150	180
α -pinene	0.71	0.88	0.80	1.68	1.08
camphene	-	t	-	-	-
β -pinene	0.21	1.64	0.45	0.65	0.65
sabinene	0.37	0.42	0.23	0.35	0.33
β -myrcene	8.70	15.46	2.96	7.43	3.58
α -terpinene	0.43	0.51	0.26	0.40	0.37
limonene	6.21	5.96	2.86	4.04	2.71
1,8 cineole	6.90	13.94	8.94	11.18	13.89
(Z)- β -ocimene	2.33	3.65	0.51	1.85	0.77
γ -terpinene	1.93	1.38	0.20	2.34	0.19
(E)- β -ocimene	0.63	0.83	t	0.44	0.56
<i>p</i> -cymene	0.83	0.62	t	0.40	t
(Z)-3-hexenol	-	0.17	-	t	t
3-octanol	0.68	0.43	t	0.45	0.32
1-octen-3-ol	0.62	0.77	0.11	0.57	0.45
menthone	2.14	0.77	4.46	1.06	1.55
menthofuran	17.43	7.45	8.17	16.27	17.87
iso-menthone	1.35	1.37	0.99	1.93	1.24
linalool	0.73	0.34	0.56	0.45	0.39
menthyl acetate	0.84	1.23	1.67	0.55	0.88
isopulegol	0.35	0.27	0.62	0.42	0.43
β -caryophyllene	0.76	4.13	4.86	3.27	2.43
neo-menthol	0.76	0.38	1.49	1.00	1.63
terpinen-4-ol	2.02	2.07	2.34	3.02	2.68
pulegone	0.55	t	0.32	t	t
menthol	22.83	22.56	32.77	27.97	32.12
(E)- β -farnesene	0.41	0.40	0.47	0.29	0.31
isomenthol	-	t	t	-	-
α -terpineol	0.61	0.44	0.52	0.47	0.53
germacrene-D	2.05	2.49	1.67	1.74	1.03
piperitone	1.16	1.19	1.81	0.67	0.56
carvone	t	0.52	t	t	0.57
β -caryophyllene oxide	t	0.28	0.27	0.12	0.42
Oil content (%)	0.17	0.20	0.23	0.27	0.37

DAT= days after transplanting

t= trace (<0.1%)

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